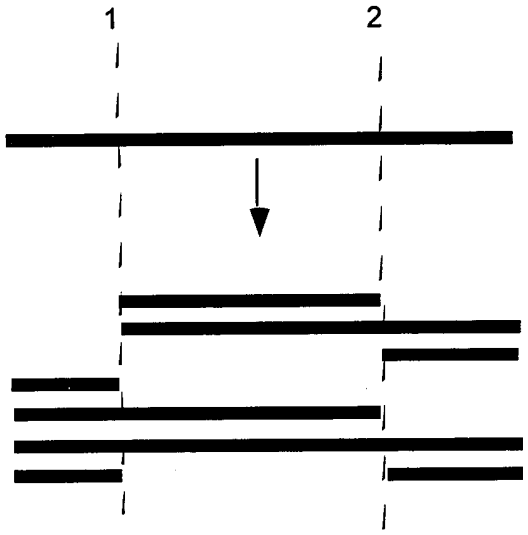
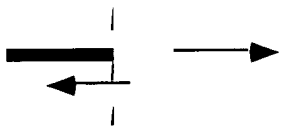
(A)

All possible recombinants prepared by crossover at positions 1 and 2



(B)

These can be prepared by assembly of synthetic fragments containing the crossover positions



Requires fragments (plus end primers):

FIG. 7

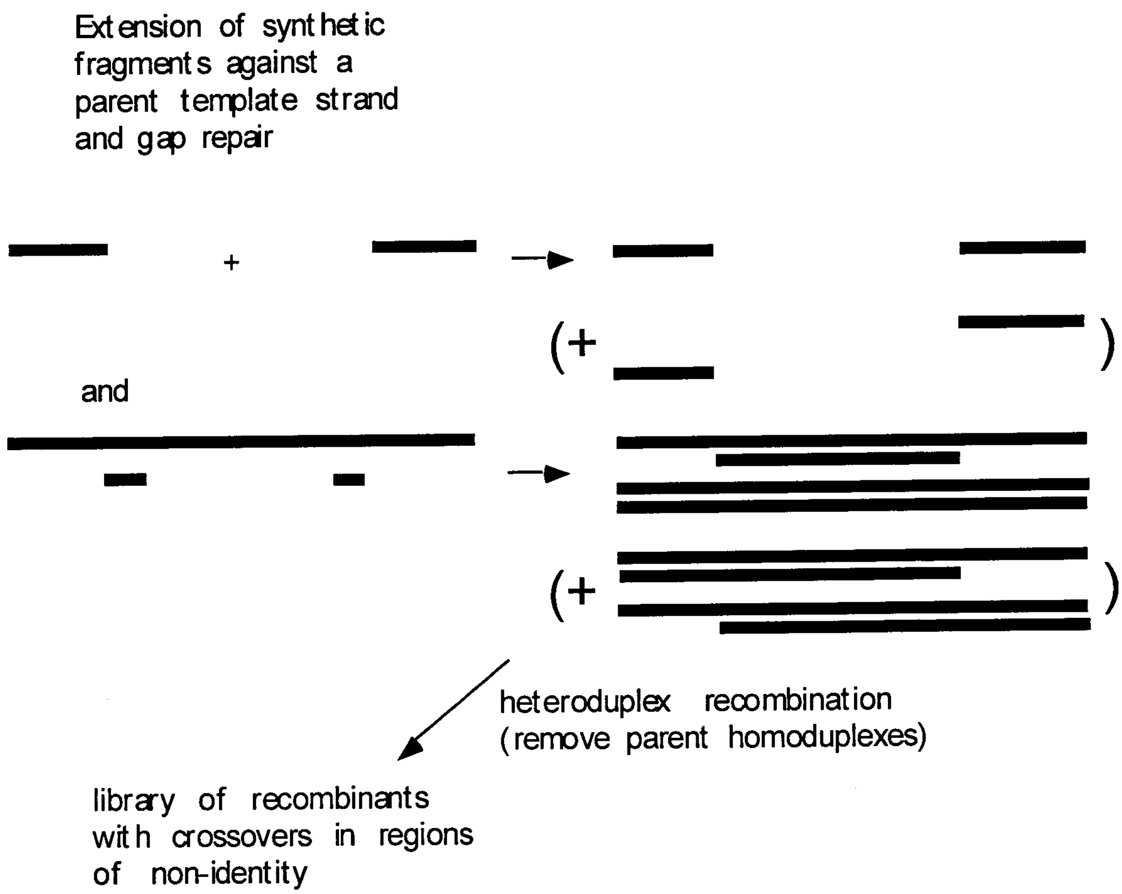
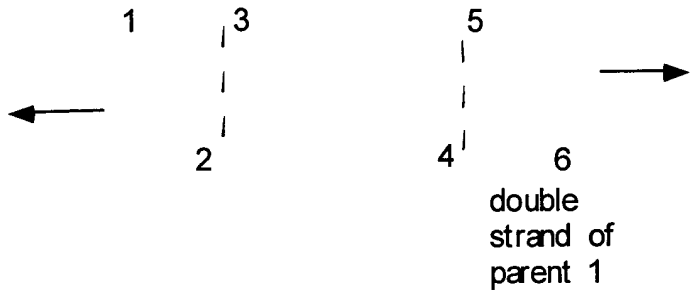

Extension of synthetic fragments against a parent template strand and gap repair
+
and
(+
)
(+
)
heteroduplex recombination (remove parent homoduplexes)
library of recombinants with crossovers in regions of non-identity

FIG. 8

(A)

Prepare the fragments by PCR with primers: perform reactions with primers 1+2, 3+4 and 5 +6,

and do same for other parent(s).



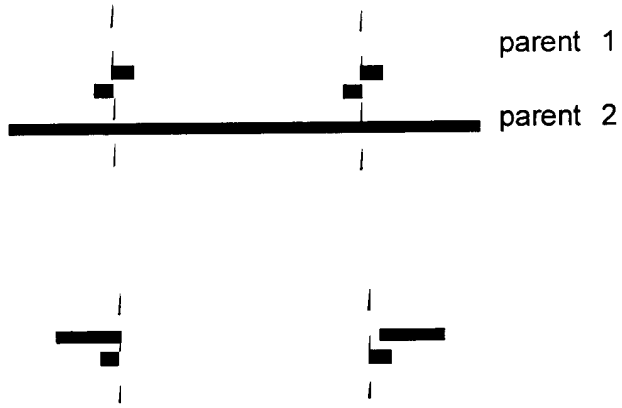
(B)

Reassemble fragments in a pool, by PCR with 1+ 6

FIG. 9

(A)

Prepare crossover primers designed to have crossovers at designated positions (2 primers for each position).



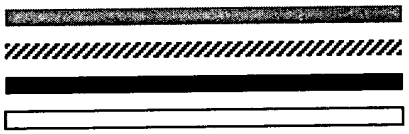
(B)

Fragment parent genes and PCR reassemble in the presence of the crossover primers to promote recombination at designated positions

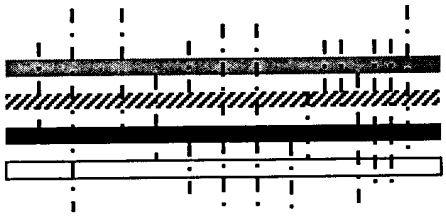
FIG. 10

Recombinant search algorithm

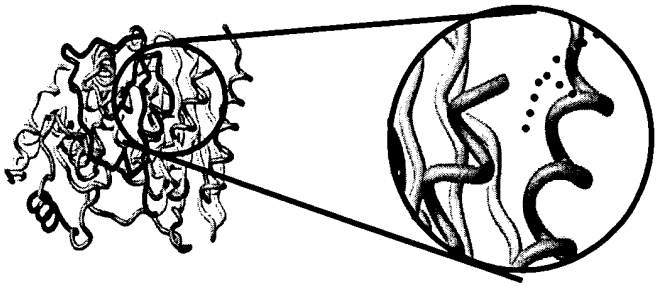
1. Align parent sequences with template structure



2. Determine all possible crossover points according to sequence identity algorithm

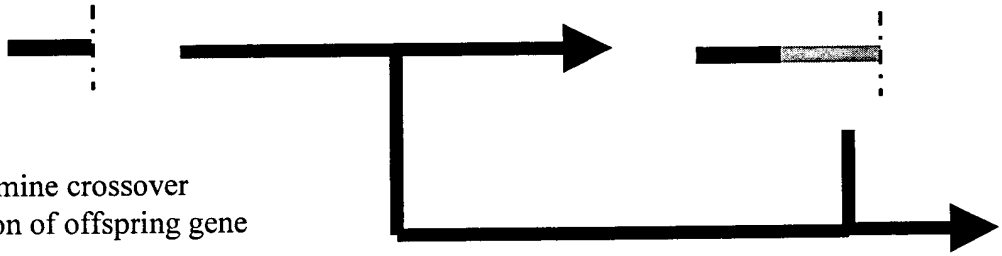


3. Calculate coupling matrix

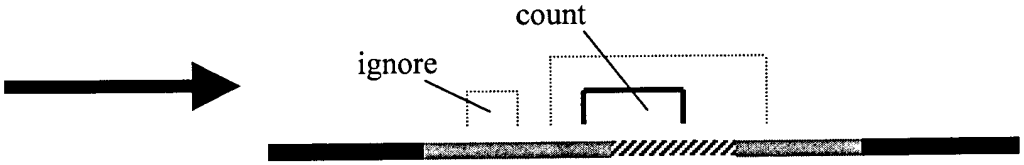


4. Pick start parent at random and copy to offspring until a possible cut point is reached

5. Pick random number, if less than *p*, copy random new parent until next cut point is reached.



6. Determine crossover disruption of offspring gene



Offspring

FIG. 12

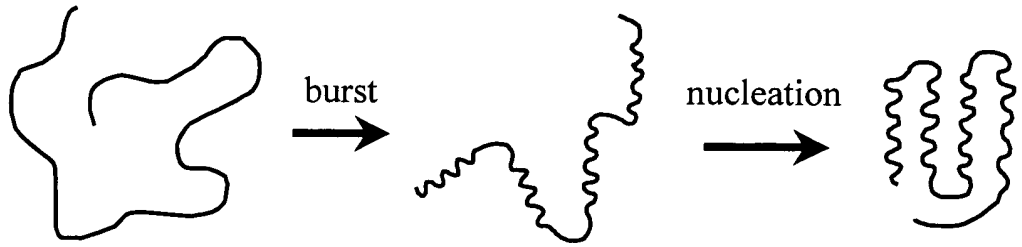


FIG. 18

The contact map shows residues that are distant (black) and residues that are close (white). If a given segment, ▬▬▬, folds an above average number of residues into a given sphere size, then it is compact.

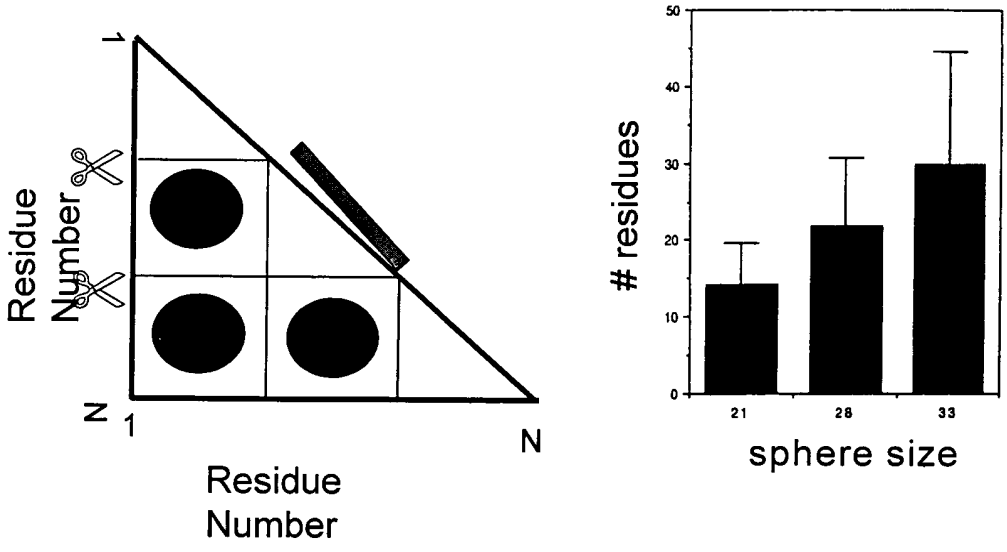


FIG. 19

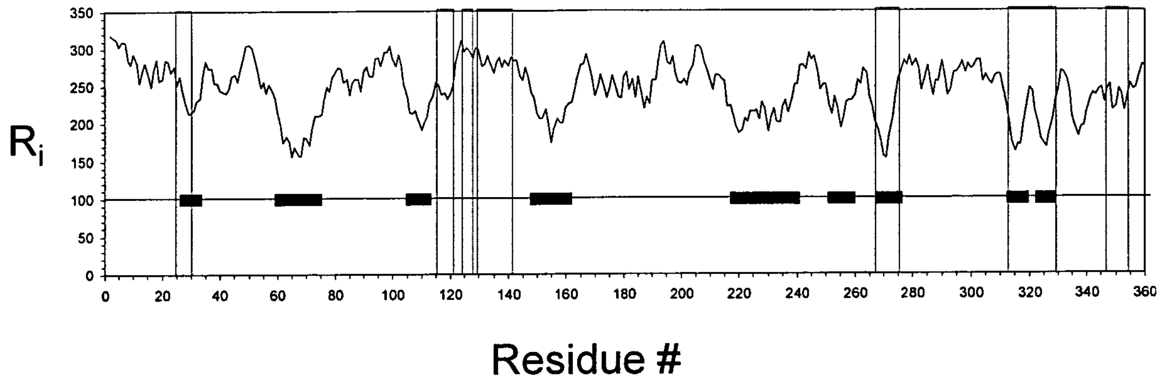
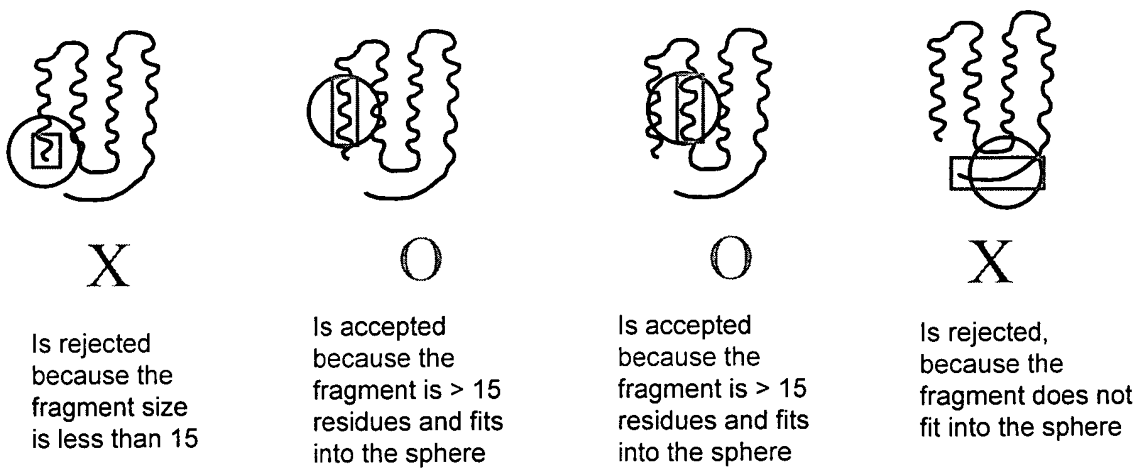


FIG. 22



(1) Pick a sphere size (21 angstroms, like Go-Gilbert) and a disruption threshold; (2) Scan protein using segments <u>at least</u> the average number of residues for that sphere size <u>or greater</u> (e.g., >15 for 21 angstrom sphere); 3) Check the disruption of all the compact fragments identified in step 2. If the fragment has a disruption above a threshold value, keep it; otherwise, throw it out; 4) If the compact unit is disruptive, increment the schema disruption measure for all of the residues in the fragment by one. This indicates that crossovers within the fragment are disfavored.

FIG. 23